On the Corpora lutea and Interstitial Tissue of the Ovary in the Marsupialia.

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With Plate 40.

Contents.

Introduction.

It is becoming increasingly evident that the secretions of the ductless glands play a very important part in determining the functions and even the structure of an animal. Not least among these are the secretions of the sexual organs, and in particular the ovary. In the mammal interest is added by the fact that ovarian secretions appear to control two very characteristic mammalian activities, namely, the preparation of the uterine wall for the attachment of the embryo, and the growth of the mammary glands (**28, 29, 30**).

According to Fränkel and Cohn (11, p. 295), "die Aplacentalia (Monotremata and Marsupialia) und die übrigen Tiere, deren Eier ausserhalb des Uterus zur Entwicklung kommen, einen rudimentären oder gar keinen gelben Körper aufweisen."

From this quotation it will be seen that some misconceptions are still prevalent regarding the corpora lutea in the marsupials, to remove which further investigation seems desirable. Moreover, as suitable material from this group of animals is difficult to obtain, it is all the more necessary that advantage should be taken of such as is available.

While looking out material for a previous investigation into the structure and origin of the corpus luteum in certain of the Marsupialia (31), Prof. J. P. Hill directed my attention to the peculiar form assumed by this body in Phascolarctos cinereus, the Australian Native Bear. Circumstances prevented me from investigating the matter more fully at the time, and it had to be laid aside for a more suitable opportunity. In the course of that inquiry, also, a well-marked glandular-looking tissue was found to be present in the ovarian stroma of certain species, the origin of which was not clear from the material then at hand, and a more extended examination was to be desired. It has since been found that the mode of formation of the corpus in Trichosurus vulpecula differs slightly from that of any other marsupial examined. Lastly, since the above-mentioned investigation was carried out, Prof. Hill has collected additional marsupial material in South America, and it is now possible to study the corpus luteum in two species of the Didelphyidæ.

Much work has been done on the corpora lutea of the Eutheria, but it is not intended to review it in any detail here, although the work of certain authors must necessarily be referred to in connection with particular points. Very good records of the literature relating to the Eutheria are already available in contributions by Sobotta (45, 46, 47), Van der Stricht (48, 49), and Marshall (24, 25). It is now perhaps generally admitted that the corpus luteum in the

Eutherian mammal is a glandular structure whose cells are the greatly modified and hypertrophied cells of the membrana granulosa of the Graafian follicle, a view expressed by Bischoff in 1842 (**6**). This statement is equally accurate when applied to the corpus luteum of the Marsupialia.

Sandes (**40**), in 1903, was the first to investigate the corpus luteum (C. L. verum) in this sub-class of the Mammalia. The material he used was obtained from pregnant Native Cats, Dasyurus viverrinus, and having access to his slides I have been able to confirm his results. The corpus luteum of D. viverrinus in which ovulation had not been followed by pregnancy was examined by me in 1912 (**32**), and I found that "at no stage is it possible to distinguish between the corpus luteum of the non-pregnant female, i.e. corpus luteum spurium, and that of the pregnant female, i.e. corpus luteum verum."

Fraenkel, in 1905 (**10**, p. 465),[1] described an ovary of Petrogale penicillata in which two corpora lutea were present. He does not deal with them in detail, as he is mainly concerned with the interstitial tissue, but his description and figure agree closely with my own findings in this species. The condition of the more recent corpus corresponds with that I have found in animals with pouch young some time after birth, although Fraenkel does not record the presence of young in the pouch.

My own previous inquiry in 1914 (**31**) into the origin and structure of this body in certain species of the Marsupialia, already referred to above, was carried out on Perameles obesula, P. nasuta, Macropus ruficollis, Petrogale penicillata, and Phascolomys mitchelli. In the first, third, and last of these corpora lutea spuria were also examined. The general result, with which that of Dasyurus is in agreement, is that the corpus luteum in all these animals resembles in all essential points that of a Eutherian mammal. The corpus luteum is a very well-marked glandular structure

[1] This isolated reference to a corpus luteum in a marsupial was not known to me when my earlier papers were written.

containing two sorts of tissue, glandular and connective. The glandular lutein cells are derived from those of the membrana granulosa mainly by simple hypertrophy, but in two cases at any rate definite evidence of a multiplication of these cells by mitosis during the formative stages was forthcoming. The connective tissue is derived from both layers of the theca folliculi, the theca interna being almost completely used up. In all cases examined it was not possible to distinguish between corpora lutea vera and spuria.

I have to thank Prof. J. P. Hill for his generosity in giving me access to his valuable and extensive collection of Marsupial material and for his criticism and advice. My thanks are further due to Mr. F. Pittock, of this College, for assistance in the preparation of the photomicrographs on Plate 40.

Material.

The source of the material has already been indicated. The ovaries of Didelphys marsupialis were nearly all fixed in picro-nitro-osmic acid, those of the remaining species for the most part in picro-corrosive-acetic acid, but one or two in Fleming's fluid or corrosive-formol-acetic acid, all of which give very good fixation. Serial sections of the ovaries about 10 μ thick were made and stained with Ehrlich's hæmatoxylin and counterstained with eosin. The sections cut for the previous investigations of Sandes (**40**) and myself (**31, 32**) were utilised for the purposes of comparison.

Phascolarctos cinereus.

Material employed.

Series.	Ovary.	Corpus luteum.	Remarks.
1	Part of ovary .	1 small old corpus luteum	Some time after the birth of young and before the ripening of new follicles.
2	Sections already cut by Prof. J. P. Hill	1 large active corpus luteum	Nothing found in uteri. Probably not long after ovulation.

Phascolarctos cinereus—(continued).

Material employed—(continued).

Series.	Ovary.	Corpus luteum.	Remarks.
3	Part of ovary .	1 large active corpus luteum, 9·25 mm. in diam.	Greatest length of embryo, 4·5 mm.
4	Ovary small, 11 × 16 mm.	1 very large corpus luteum at one end, 11 mm. in diam.	Greatest length of embryo, 7·5 mm.
5	Ovary irregular, 15·25 × 10·5 mm.	1 large corpus luteum at one end, 10 mm. in diam.	Greatest length of embryo, 9 mm.
6	Ovary, 13·5 × 10 mm.	1 large corpus luteum, 9·75 mm. in diam.	Greatest length of embryo, 11 mm.
7	Ovary, 12 × 8·5 mm.	1 active corpus luteum, 8·5 mm. in diam.	Greatest length of embryo, 12·25 mm.
8	Ovary, 13 × 10 mm.	1 large corpus luteum forming nearly whole of ovary, 10 mm. in diam.	Greatest length of embryo, 13 mm.
9	Ovary very large, 19·5 × 11·5 mm.	1 large corpus luteum at one end, 10 mm. in diam.	Moderately advanced uterine embryo, length not ascertained.
10	Ovary long, 15 × 7·5 mm.	1 active corpus luteum at one end, 7·5 mm. in diam.	Greatest length of embryo, 17 mm.
11	Ovary irregular, 16 × 9·5 mm.	1 active corpus luteum, not a large part of ovary, 8 mm. in diam.	Newly born young. Greatest length, 16·5 mm.
12	Ovary triangular, irregular, 17 × 14 × 13 mm.	1 active corpus luteum, not a large part of ovary, 8 mm. in diam.	Shortly after birth of young.

The ovary of Phascolarctos is quite a large body, in some cases reaching a length of more than 19 mm., and is more or less smooth. After ovulation it possesses a single active corpus luteum which forms a large part, sometimes almost the whole, of the ovary. In no case was more than one active corpus luteum found in the same ovary, so that only one follicle bursts at a time. In correlation with this it is to be noted that generally only one uterus becomes pregnant for in the records available there is no instance of a female

with both uteri pregnant. The corpus luteum is conspicuous throughout pregnancy, and the point of rupture of the follicle from which it was derived is visible for a long time. The ovary in a resting condition (series 1) still has present in it remains of a previously active corpus luteum, and in all of the remaining ovaries a similar structure is to be found. In spite of the fact that the corpus luteum in this animal probably persists for a long time, the presence of an old one at the same time as an active one seems to indicate beyond doubt that there is more than one ovulation period in the year. It is then unlike Dasyurus (16), and may be polyœstrous with several ovulations following one another, or, perhaps more probably, has two or more breeding periods in the year and the corpora lutea persist for a comparatively long time, an interpretation that is borne out by the fact that in some cases two old corpora are present. The same is supported by the finding of follicles beginning to ripen in the ovaries of Nos. 11 and 12 just after the birth of the young.

The Follicular Wall.—The structure of the Graafian follicle and its formation agree closely with that in Dasyurus and other marsupials. No quite ripe follicle was examined, although nearly ripe follicles are present in series 11 and 12. These are distinguishable not only by their size, but by the fact that the membrana granulosa is reduced to three or four cells deep and that no indication of mitoses is to be found in its cells, although mitotic figures are common in them during the earlier stages of the follicle. The ovum in its discus proligerus is situated to one side and the central cavity is filled with the coagulum of the liquor folliculi. The outer limit of the membrana granulosa is marked by the membrana propria, and this homogeneous basement membrane shows clearly at the points where the granulosa cells have shrunk away from it. The theca folliculi is well marked, and its cells readily distinguishable from those of the membrana granulosa. It is divisible into a theca interna of somewhat flattened polygonal cells and a theca externa of greatly

elongated cells which grades off into the surrounding ovarian stroma. Careful searching failed to reveal the inclusion of any interstitial cells in either layer of the theca.

The Formation of the Corpus Luteum.—Unfortunately no stage immediately after the rupture of the follicle was available, the earliest being No. 2 (Pl. 40, fig. 1) in which the corpus luteum is already definitely established. In this female the uteri were slightly enlarged, but nothing was found in them, a fact that may indicate that the animal was now pregnant or that the ova had been overlooked. It is young enough, however, to indicate clearly that the method of formation resembles that met with in Perameles obesula, P. nasuta, and in Macropus ruficollis (31). The connective tissue of the theca folliculi bursts through the membrana granulosa and quickly spreads out to form an internal layer surrounding the central cavity, the cavity itself being filled with a denser coagulum than that in the follicle (Pl. 40, fig. 2). This differs from the condition in Dasyurus viverrinus (40), where the connective tissue does not form an inner layer, but goes straight into the cavity and commences to form an aggregation in the centre.

Mitoses are present in this early corpus luteum, but in all cases they are distinctly in connective tissue cells and not in the lutein cells themselves. There is no evidence, therefore, that these cells multiply mitotically, although they may do so in an earlier stage. In the formation of the ingrowths both layers of the theca folliculi take part, and the inner one is almost entirely used up; it is possible that some of its cells may remain in their original position, but they are few in number. The theca cells are at all times totally unlike the cells of the membrana granulosa in situ or when they are transformed into lutein cells, and there is no indication that any of them are modified to form the latter. Accompanying the thecal irruptions, which sometimes run straight through to the internal connective tissue layer, are blood-vessels coming from those of the theca externa.

The lutein cells are derived entirely from the cells of the

membrana granulosa, which with their nuclei undergo enormous hypertrophy and soon begin to secrete actively.

The fully formed Corpus Luteum.—On the whole the stage of the corpus luteum corresponds to the stage of the embryo in the uterus, that is to say, the older corpora lutea are found in the animals with older embryos. No. 3 offers a notable exception to this, for, although the embryo is quite young, the corpus luteum is at a stage of development corresponding with that in an animal in which the young are born. This may indicate that ovulation is independent of copulation and consequent fertilisation, but the evidence is insufficient to enable one to assert that such is the case.

It has just been pointed out that in two species of Perameles and in M. ruficollis the corpus luteum is formed as a hollow structure, but it only remains so in the very early stages, and the central cavity entirely disappears soon after the embryo has acquired one or two somites. In Phascolarctos cinereus this hollow condition persists throughout the period of pregnancy, and, although the cavity gradually gets reduced in size, it is always present, so that a section of the corpus luteum presents a very striking appearance (Pl. 40, fig. 3). Even after the birth of the young, as, for example, in Nos. 11 and 12, the central cavity is still obvious enough, although the internal layer of connective tissue has become stellate instead of circular. Whether or not this hollow condition has any significance either functionally or phylogenetically it is not yet possible to say, but the fact remains that it produces a corpus luteum which, during the time that the embryo is in the uterus, is quite unlike the corpus in any other marsupial that has been examined. Indeed, as far as I am aware, its structure is unique, and has not been observed in any other mammal.

An interesting phenomenon calls for notice in connection with this form of the corpus luteum. Prof. J. P. Hill informs me that in all the genital tracts of marsupials that he has handled, some hundreds in number, it is only in P. cinereus that he has encountered cystic ovaries, and in this species

such a condition is by no means uncommon. Moreover, the cystic condition is not confined to the ovary; it may extend to the Fallopian tube and uterus. Whether the hollow corpora lutea favour the formation of cysts in the ovaries, or whether there is any relation between the two, is not clear, but the occurrence of cystic ovaries in this species far more frequently than in any other is certainly worthy of record.

An old corpus luteum in an ovary that also has an active one is readily distinguishable from the latter by the absence of the central cavity. In addition to this it is smaller in bulk, its lutein cells are smaller and far less active, and the theca folliculi is not so sharply marked off from the surrounding stroma. The material at my disposal does not show at what period the central cavity is lost, but it is certainly some time after the birth of the young.

There is also present in the ovary, besides the active corpus luteum and one or perhaps two old corpora, a fair amount of interstitial tissue scattered in moderate-sized masses throughout the stroma. This, however, will be more fully dealt with later, and here it is only necessary to call attention to its presence.

Trichosurus vulpecula.

Material employed.

Series.	Ovary.	Corpus luteum.	Remarks.
1	Ovary, 8 × 4·25 mm.	1 small corpus luteum, 3 × 2 mm.	These are from animals apparently not long after ovulation, that may or may not have been pregnant; but no records are available.
2	Large ovary, 10·5 × 6 mm.	1 small corpus luteum, 2·5 mm. in diam. Probably recently ruptured	
3	Large ovary, 9·5 × 6 mm.	1 corpus luteum, 4 × 3 mm.	
4	Ovary, 8×5 mm.	1 corpus luteum, 3 × 3 mm.	
5	Ovary, 9×6 mm.	1 corpus luteum, 4 × 3·5 mm.	
6	(*a*) Right ovary, 6 × 9 mm.	1 old corpus luteum, 3 × 2·75 mm.	Left ovary not long after ovulation, but no record available.
	(*b*) Left ovary, 9 × 5 mm.	1 corpus luteum, 2·25 mm. in diam.	

Trichosurus vulpecula—(continued).

Material employed—(continued).

Series	Ovary.	Corpus luteum.	Remarks.
7	Ovary, 8×5 mm.	1 corpus luteum, 4 × 2·5 mm.	Early ovum.
8	Ovary, 7·5×4.75 mm.	1 corpus luteum, 4 × 2·25 mm.	Small blastodermic vesicle, 1·6 × 1·4 mm.
9	Small ovary, 6·75 × 6 mm.	1 corpus luteum, 3·25 × 3 mm.	Blastodermic vesicle, 1·8 mm. in diameter.
10	Ovary, 7×5 mm.	1 corpus luteum, 4·75 × 5·25 mm.	Blastodermic vesicle, 6 mm. diam.
11	Small ovary, 6×5 mm.	1 large corpus luteum, 6 × 4·5 mm.	Larger blastodermic vesicle.
12	Ovary, 7·25 × 6 mm.	1 large corpus luteum, 7 × 4 mm. Top widely expanded	Slightly older than 11 stage γ.
13	(*a*) Right ovary, 6·5 × 6 mm. (*b*) Left ovary, 8 × 7·5 mm.	1 large corpus luteum, 5·75 × 6·25 mm. 1 old corpus luteum, 2·75 diam.	Greatest length of embryo, 11·5 mm.
14	Ovary, 7 × 4·5 mm.	1 corpus luteum, 4 × 2·25 mm.	Greatest length of embryo, 14·5 mm.

Trichosurus vulpecula is a smaller animal than Phascolarctos and the ovary is correspondingly smaller, the largest measuring 10·5 × 6 mm. In this, as in the previous animal, there was never more than one active corpus luteum in the ovary, showing that only one follicle bursts at a time. Also only one uterus was pregnant in each animal. Furthermore, where the two ovaries from the same female were examined, it was seen that, although each contained a corpus luteum, they were not of the same age. That on the side of the pregnant uterus was an active corpus, while that in the other was older. Often in the same ovary with the active yellow body was another still older than that of the opposite ovary. These facts seem to indicate clearly that the ovaries ovulate alternately and, further, that one ovulation period follows another after a moderately short interval. The female then has undoubtedly more than one œstral period in a year, a point that is further indicated by the

fact that the ovaries with older corpora lutea usually contain several follicles approaching maturity.

The Follicular Wall.—As before, no absolutely ripe follicle was examined, but in Nos. 6, 8, and 9 well-advanced follicles were obtained. The description already given for Phascolarctos will apply equally well to Trichosurus. The main and perhaps only noticeable difference between the two species is that of size, and the structure of the follicular wall and its component cells is the same in the two cases.

The Formation of the Corpus Luteum.—No example shows the follicle immediately after the expulsion of the ovum, though several exhibit very early stages in the formation of the corpora lutea. Nos. 1–5 are all early stages, i.e. soon after ovulation, as is shown by the small size of the corpora lutea and also by the fact that in none of them are the other follicles nearly ripe. Nothing was found in the slightly enlarged uteri of these animals, so that here again we are dealing with females that were either not pregnant, or, more probably, pregnant and the ova overlooked on account of their small size, a very likely occurrence when the fixation is done in the field.

In No. 2 (Pl. 40, fig. 4), the corpus luteum is quite young, as the transforming cells of the membrana granulosa are not much enlarged and are only five or six cells deep. It indicates that the rupture of the follicle is entirely different from that of all the marsupials previously described. In all the others the rupture takes place at a definite point, which is almost immediately closed by a plug of cells (Bouchon épithélial), so that immediately after the egg has been discharged there is still a large central cavity, though it is probably somewhat smaller than that of the ripe follicle. The connective tissue of the theca then bursts in at a number of places and runs in strands more or less radially towards the centre, afterwards forming a network through the lutein cells. The follicle in Trichosurus vulpecula, however, when it bursts, collapses altogether, and its walls are brought together so that the central cavity is entirely obliterated

(Pl. 40, fig. 5), save for isolated portions that may remain here and there closed in by folds of the membrana granulosa. Remains of the hollow shut in in this way are very well displayed in No. 11. At the same time the theca is, of course, drawn in by the collapsing follicular walls, and in consequence large irregular masses of connective tissue are to be found in between the forming luteal cells. In some places it appears to burst right through the membrana granulosa and help form a plug of connective tissue and membrana cells, which closes the actual point of rupture. The result is that an extremely irregular structure is formed (Pl. 40, fig. 6). Mitoses were not found in the young luteal cells in any of the early stages. The connective tissue ingrowths, as in other species, are derived from both layers of the theca folliculi, but the folding of the wall of the follicle consequent upon its collapse brings in some of the connective tissue of the ovarian stroma that would be considered outside the actual theca externa. In spite of this no evidence of the inclusion of interstitial cells was obtained.

A similar method of formation of the corpus luteum in which the follicle collapses on rupture is described by Sobotta (**44**) in the mouse, but does not appear to be common even in the Eutheria.

The fully formed Corpus Luteum.—The corpus luteum from the stage just described increases enormously in size and reaches its maximum shortly after the embryo has passed through the blastodermic vesicle stage, i.e. in Nos. 11 and 12, in the latter of which it measures 7 × 4 mm. The fully grown corpus luteum is much more irregular than in the other marsupials so far examined, and is further unlike them in not possessing a central plug of connective tissue (Pl. 40, figs. 5, 6, and 7). The very large bands of connective tissue cut the body up into a number of parts, and there is not the slightest suggestion of a radial arrangement of the main strands of the connective tissue. Although so irregular it is more or less bound together by a fibrous sheath to form one whole body. Owing to the way in

which the follicle bursts it sometimes happens, as in Nos. 6 (Pl. 40, fig. 6) and 12 (Pl. 40, fig. 7), that it is partly inverted and that the lutein cells become pushed out and a mushroom-shaped corpus luteum is produced. These cells, however, are quickly covered by a layer of connective tissue. Such cases are interesting from the light they throw on the origin of the lutein cells. Here, if anywhere, among the Marsupialia was an opportunity for shedding the membrana granulosa, but not only is this not done, but the membrana cells still remain adherent to the theca folliculi even in the inverted portion of the lips of the follicle. Some of these cells are very probably lost, but by far the larger number remain and give rise to lutein cells. The marsupial ovary offers marked advantages for the observation of these changes, for, as has already been pointed out, there is always a marked difference between the cells of the theca interna and those of the membrana granulosa. There is no doubt whatever that throughout this group the theca interna plays no part in the production of lutein cells, these being, on the contrary, all derived from the membrana granulosa. It is necessary to insist on this point, as several fairly recent writers, for example, Hegar (**15**), Pottet (**35**), and Delestre (**9**), in certain Eutherian mammals support the theory of von Baer (**4**), who maintained that the lutein cells originate solely from the theca interna, while the membrana granulosa cells were either discharged with the ovum or degenerated immediately afterwards. It does not seem probable that the corpus luteum, which is practically identical in the two groups, should arise in such different ways, and, moreover, a number of careful observers, including Sobotta (**44–47**), Van der Stricht (**48, 49**), Loeb (**22**), and Marshall (**23**), have maintained that in the Eutheria the lutein cells come from the membrana granulosa. The only feasible explanations seem to be that the accounts given by these supporters of von Baer rest upon faulty technique, an incomplete series of stages, or misinterpretation of the sections.

Didelphys aurita.

Material employed.

Series.	Ovary.	Corpus luteum.	Remarks.
1	Small, 7 × 6 mm.	Points of rupture still visible	Unsegmented eggs.
2	Small, 8 × 5 mm.	Corpora lutea small	Segmentation stage; 2- and 3-celled.
3	Small, 8 × 6 mm.	Corpora lutea small	Segmentation stage.
4	Small, 8 × 5 mm.	Corpora lutea small	Segmentation stage.
5	Larger, irregular, 10 × 6 mm.	Corpora lutea large	Eggs in a late segmentation stage, but abnormal.
6	Irregular, 9 × 8 mm.	Corpora lutea large	Blastodermic vesicles, ·86–1·1 mm. in diam.
7	Irregular, 9 × 7 mm.	Corpora lutea large	Blastodermic vesicles, 1·3–1·5 mm. in diam.
8	Small, 7 × 5 mm.	Corpora lutea not visible externally	Pouch young.

This animal differs considerably from the foregoing two and agrees with Dasyurus in that a large number of eggs, 12–14 (in one case 19), may be discharged simultaneously from each ovary. Selenka (**43**, p. 104) states that normally the opossum D. marsupialis breeds only once in the year, but in certain cases may experience œstrus again at the latest at the beginning of June. In Brazil, however, Prof. J. P. Hill found that there were at least two breeding seasons in D. aurita, pregnant females being obtained in July and again in October, so that either this is a point of difference between the species or Selenka's statement may require modification.

D. aurita, therefore, may be polyœstrous, or, more probably, it may have several breeding seasons in the year. The corpora lutea persist throughout pregnancy, but not long afterwards, and in no case where active corpora lutea are present are corpora fibrosa to be found. In the example, with fairly well-grown pouch young, the corpora lutea have

already nearly disappeared. The ovary resembles very closely that of Dasyurus in appearance, and is of moderate size, the largest examined measuring 9 × 8 mm.

The Follicular Wall.—Fairly ripe follicles are present in No. 1, in which the structure of the walls can be readily made out. The basement membrane is well marked, and the theca folliculi differentiated into an external and internal layer. The theca interna is not so clearly marked off from the outer layer as in the macropods, and is similar to that in Dasyurus viverrinus, and both layers are readily distinguishable from the membrana granulosa cells. The latter form a layer three or four cells deep on the inner side of the basement membrane.

The Formation of the Corpus Luteum.—This series is fairly rich in early stages, for no less than four animals yielded eggs in various stages of segmentation before the formation of a blastodermic vesicle. The membrana granulosa is not thrown off when the follicle bursts, though doubtless a little of it is lost in the very superficially situated follicles, and the point of rupture is soon closed. This has already occurred in the earliest example (No. 1), where the membrana granulosa cells have started to enlarge and are arranged around the young corpus luteum 5–9 deep. On ovulation the basement membrane is broken through in a number of places by the thecal ingrowths and their accompanying blood-vessels. They do not burst through the membrana granulosa until later, but instead they push the transforming membrana in front of them, and consequently this appears plicated in section (Pl. 40, fig. 9). The central cavity thus assumes a stellate form, which, however, is not to be confused with the stellate plug of connective tissue that is to be found in other species, e.g. Phascolomys mitchelli (**31**). It is still the central cavity and contains only a coagulum, the remains of the liquor folliculi, and perhaps one or two blood-corpuscles and phagocytes let in as a result of the rupture. This cavity is still lined with the membrana granulosa cells in course of transformation

into lutein elements, and not by a layer of connective tissue as in the case of Phascolarctos cinereus, Perameles, and certain macropods (31). No. 1 in the series is further interesting, as mitoses are to be found in the transforming membrana cells, thus indicating clearly that in the very early stages these cells multiply by mitosis. Evidence of a similar multiplication by karyokinesis has been brought forward in the case of Perameles obesula and P. nasuta (31). The later stages of Trichosurus do not show these mitotic figures in the lutein cells, and as they are never plentiful even in the early stages, it would appear that although hypertrophying young lutein cells do multiply, it is not to a very great extent.

The later formation stages to be found in Nos. 2, 3, and 4 show that the enlargement of the lutein cells gradually reduces the central cavity (Pl. 40, fig. 10), and it is not until it is almost obliterated that the connective tissue sprouts break through and form the central plug (Pl. 40, fig. 11). It is to be noted that where the follicles are situated right on the periphery of the ovary the rupture is followed by a complete collapse of the follicle, and consequently the central cavity disappears right from the beginning. This occurs normally in T. vulpec, as just described.

The fully formed Corpus Luteum.—The fully formed corpus luteum which is found in Nos. 5 (Pl. 40, fig. 12) and 6 calls for little comment, as it is typical in all respects, and it resembles most nearly those of Dasyurus (40) and Perameles. The connective tissue in it is very well developed and ramifies throughout the entire corpus, accompanied by small blood-vessels. The large number of follicles bursting at the same time leads to the formation of a corresponding number of corpora lutea, and when they are fully grown the ovary is mainly composed of these glandular bodies (Pl. 40, fig. 8), the stroma with the remaining young and primordial follicles being reduced to a minimum.

The old Corpus Luteum.—No. 8 had young some weeks after birth, and in the ovaries traces of old corpora

lutea were to be seen. They were much reduced in size, and the cytoplasm and nuclei of the individual cells were in an advanced stage of degeneration. Phagocytes are plentiful and appear to be concerned with the removal of the old lutein cells much in the same way as Sandes (**40**) has described in D. viverrinus. The connective tissue sheath of the corpus is very indistinct and hardly to be distinguished from the surrounding stroma. This animal was taken in the earlier breeding season of the year, and there is no doubt that the corpora lutea would have completely disappeared before the onset of the next. That this would not occur immediately is indicated by the development of the follicles which are not yet ripe.

Material from Further Species.

In connection with the question of interstitial tissue, to be dealt with shortly, a number of other ovaries of different species were cut and examined, but in no animal were early stages in the corpus luteum formation obtained. All the ovaries possessed corpora lutea vera, but as they do not offer any special points of interest they may be dealt with quite briefly.

Metachirus nudicaudatus.—The ovaries were small, squarish, and fairly smooth, measuring 6 × 5 mm. Early blastodermic vesicles were present in the uteri. The corpora lutea, of which there were several in each ovary, were fully formed, of small size, and their blood-vessels well distended with blood. In transverse section the ovary is very similar in appearance to that of D. aurita and Dasyurus viverrinus. No interstitial tissue was found.

Macropus ualabatus.—This female was in the later stages of pregnancy. The ovaries were large, 11·25 × 6 mm., and contained a single corpus luteum 6 mm. in diameter situated at one end and clearly marked off from the remainder of the ovary. In transverse section the ovary and corpus luteum were similar to that of macropods previously described,

and a fair amount of interstitial tissue is scattered in small islands throughout the stroma. The lutein cells were full of droplets of fatty secretion, dissolved out in preparing the sections, indicating that the gland was secreting actively.

Macropus dorsalis.—The animal was killed shortly after parturition. The ovary contains one large active corpus luteum and two older ones (Pl. 40, fig. 13). A fair amount of interstitial tissue is present, differing in appearance from both the active and old corpora lutea and situated in one part of the ovary, although split up into a number of smaller separate masses. The whole ovary is similar to that of the other macropods and measures 10 × 6·25 mm. The active corpus is situated towards one end and is 6·25 mm. in diameter.

Macropus thetidis.—The ovary of this pregnant female was smallish, 7 × 4·5 mm., and the major portion of it is occupied by two large fully grown corpora lutea and a small amount of interstitial tissue. The general appearance is that of a typical macropod ovary.

Macropus parma.—Here again we are dealing with the ovary of a typical macropod. It is irregular, 8·5 × 6·5 c.c., and its bulk is mainly taken up by a single prominent corpus luteum and a large amount of interstitial tissue. The uteri contained embryos in the blastocyst stage, but the corpus luteum appears to be fully hypertrophied.

Onychogale frenata. (The species is somewhat doubtful. It is undoubtedly a macropod and probably O. frenata.)—The ovary was large, smooth, and roundish, and measured 10 × 10 mm. It contained two corpora lutea, one apparently slightly younger than the other, but both fully formed. Although the embryos were in an early blastodermic vesicle stage, the corpora appear about fully grown. A little interstitial tissue is present in the form of small scattered groups of cells, and the transverse section of the whole ovary is very similar to that of M. ualabatus.

The Corpus Luteum in the Marsupialia.

A certain number of questions have arisen in regard to the structure and histogenesis of the corpus luteum in the Eutheria: whether the lutein cells are derived from the cells of the membrana granulosa or of the theca interna wholly or in part; whether these cells increase by mitotic or amitotic division; what are the precise parts played by the theca interna and theca externa in the formation of the connective tissue network; whether any difference exists between the corpus luteum in the pregnant and non-pregnant animal, and so on. It is not my intention to enter into a discussion of the evidence that has been adduced on both sides in these matters, as that has already been dealt with briefly elsewhere (**31**), and subsequent investigations have only confirmed the conclusions set forth in that review. Now, however, when a fairly representative collection of ovaries has been examined, it does seem advisable to review briefly the main characteristics of the corpus luteum in the Marsupialia. This may be done the more readily as all the fifteen species so far described have been cut and examined by myself, and moreover, the sections cut by Sandes for his work on the pregnant Dasyurus viverrinus are in the possession of Prof. J. P. Hill, who has kindly handed them to me for examination. It is a distinct advantage for the purposes of comparison that almost without exception the material was very well fixed and preserved, and that a fairly uniform technique in cutting and staining has been adopted throughout. In all cases, too, serial sections have been employed. Perhaps the first point to which attention should be directed is the striking similarity between the corpus luteum of the Marsupial and the Eutherian. Indeed, I know of no criterion whereby the structures in the two classes can be distinguished from one another. The statement of Fraenkel and Cohn (**11**) given in the introduction, and repeated without criticism by Van der Stricht ('Archiv. de Biol.,' t. xxvii, 1912, p. 586), is therefore without foundation in fact, for the marsupial ovary, if examined at the right

time, always possesses a well-marked corpus luteum. Minor differences in appearance are to be found in various species as they are in the Eutheria, and although the corpus in Phascolarctos cinereus is different from that in any higher mammal yet described, it also differs in the same way from that in any other marsupial. Generally, then, the histological structure and appearance of the gland, and also its size relative to the follicle or ovary, as a whole is the same in the two groups.

The Graafian Follicle.—The follicle is very similar throughout, and when ripe or nearly ripe consists of a large central cavity filled with liquor folliculi and surrounded by the membrana granulosa, to one point of which is attached the ovum in a discus proligerus. The membrana granulosa is composed of small polygonal cells from three to five deep, with spherical nuclei and moderately clear cytoplasm, and in which mitotic figures are uniformly absent. Its outer limit is marked by a clear homogeneous membrane—the membrana propria—and the whole is similar to the same structures in the Eutheria. The theca folliculi is, on the whole, perhaps, not quite so distinct as in the higher mammals, but always divisible into internal and external layers. The theca interna varies slightly in its development in different species, and it is best marked in Phascolomys wombat, where it is composed of longish polygonal cells three or four deep lying immediately outside the membrana propria. Its development in this species most closely approaches that met with in the Eutheria, but it is relatively thinner and its cells more elongated. A very similar condition obtains in Phascolarctos cinereus The macropods and Trichosurus vulpecula do not possess such a well-marked theca interna, and it is still less developed in the two species of Perameles in Metachirus nudicaudatus and Didelphys aurita. The extreme is met with in Dasyurus maculatus and viverrinus, where, although easily recognisable in a young follicle, this layer is almost indistinguishable from the theca externa in the ripe follicle. It is further important to notice

that even in those species where it reaches its highest state of development the cells of the theca interna are very different from those of the membrana granulosa, so that the subsequent history of the two layers can be followed with more certainty than in the Eutheria. No evidence of the inclusion of interstitial cells was forthcoming in any case. The theca externa calls for no comment, as its fibrous cells are similar in the different species and again in the higher mammals.

The Rupture of the Follicle.—With one exception, Trichosurus vulpecula, when the follicle bursts it discharges the ovum and almost immediately closes again, either by the formation of a sort of plug of connective tissue and lutein cells (Bouchon épithélial) or by the coming together of its walls. In Trichosurus, as just described, the follicle completely collapses, a phenomenon recorded also in the mouse. The very superficially situated follicles in Didelphys aurita collapse in a similar manner, but not so irregularly. The membrana granulosa is always retained at ovulation, although it is possible that a few of its cells may be lost at the actual point of rupture.

The Formation of the Corpus Luteum.—In most species, immediately after ovulation the theca folliculi, accompanied by blood-vessels, breaks through the membrana propria and then through the membrana granulosa. Both theca interna and externa take part in these irruptions, and the former is almost completely used up in the process. In Perameles obesula and nasuta, Macropus ruficollis and P. cinereus, the irruptions quickly spread out on the inner side of the membrana granulosa to make a sort of inner connective tissue sheath. This does not happen in D. viverrinus, D. aurita, and T. vulpecula. The connective tissue sprouts in the first of these break through and form a loose central plug of tissue; the same occurs in the second, though in this case the membrana granulosa remains intact for a longer time, its transforming cells are pushed inwards by the ingrowths. The collapse of the follicle in the third of these species is so complete that the irruptions break

through in a very irregular manner and are of such size that they may even carry some of the surrounding fibrous tissue with them. The cells of the thecal ingrowths multiply by mitosis in the early stages.

Shortly after the rupture, the cells of the membrana granulosa and their nuclei commence to hypertrophy and to undergo modification into lutein cells. Definite evidence of mitotic division of these cells has been obtained in P. obesula and nasuta, and also D. aurita. In the opinion of the observer the multiplication of the membrana granulosa cells is not of great extent and confined to the very early stages, and this accounts for the fact that mitotic figures have not been observed in other species.

The fully formed Corpus Luteum.—The corpus luteum is formed when the embryo is in the early blastodermic vesicle stage, and reaches its maximum size a little later. It is a solid body, except in P. cinereus, where it is hollow, composed of a mass of lutein cells surrounding a central plug of connective tissue. Between the cells ramifies a network of the same tissue accompanied by blood-vessels serving for nutriment and perhaps support. The central plug is absent in the corpus of T. vulpecula, which presents a much less regular appearance in section than any of the others. The whole structure is separated from the ovarian stroma by a fibrous sheath, the remains of the theca externa. The lutein cells are derived entirely from the cells of the membrana granulosa, and, although similar throughout, are slightly less compressed and have slightly clearer nuclei in D. viverrinus, P. obesula and nasuta, D. aurita and M. nudicaudatus, than in the remaining species. The connective tissue network is derived from both layers of the theca folliculi, and ramifies through the cells slightly more intimately in the just-mentioned species than in the others. The result is that the corpora lutea in these species resemble one another more closely than they do the corpora of the other species, but the difference is only small. It is interesting to note that the ovary and corpus luteum of the South American form D. aurita most

closely approach those of the Australian D. viverrinus, and thus confirm the near relationship between them that is indicated by other anatomical and developmental features.

The Corpus Luteum Spurium.—Ovaries of non-pregnant females containing corpora lutea have been examined in D. viverrinus, P. obesula, P. mitchelli, and Petrogale penicillata, and in each case they were found to be identical in size, structure, and appearance with corpora lutea vera. Further, in the first two species their mode of formation was also studied and found to be the same as in the pregnant female. The formation and structure of the corpus luteum then, is not influenced by the subsequent fate of the ovum, that is whether it is fertilised or not, and in the case of D. viverrinus the duration of the two bodies was found to be about the same (**32**).

The Interstitial Tissue.

In both P. cinereus and T. vulpecula, described above, there is present in the ovary a quantity of tissue embedded in the stroma that is not to be found in D. aurita. It is particularly plentiful in the first-mentioned species. Similar tissue was found in certain other marsupials previously examined, about some of which it was stated: "Die Ovarien . . . enthalten in ihrem Stroma eine mässig grosse oder auch grosse Menge eines Gewebes, welches dem Gewebe der interstitiellen Drüse bei den Ovarien einiger höherer Säugetiere gleicht" (**31**, p. 28). At that time it was not possible to say more, owing to insufficiency of material for comparison, and so it is proposed to enter a little more fully into the matter here.

These interstitial cells have received a great deal of attention from various authors, and good reviews of their work are to be found in the papers of v. d. Stricht (**49**) and Schaeffer (**41**). But, so far as I am aware, only four authors, Benthin (**5**), Fraenkel (**10**), V. d. Broek (**7a**), and Schaeffer (loc. cit.), have examined this tissue in the mar-

supial ovary, and their results will be discussed later. Interstitial tissue is not universally present in the mammalian ovary; a number of species do not possess any trace of it, but it is very widespread throughout the entire class, and is often present in large quantities. Various theories as to its origin have been put forward, but no general agreement has yet been arrived at.

Paladino (33) attributes an epithelial origin to these cells, and this was also maintained by certain earlier authors, Nussbaum (27), Harz (14), etc., but the evidence in support of this view is unsatisfactory. Lane-Claypon (19, 20) describes them as originating in the rabbit as sister cells of the ova, and, therefore, to be regarded as potential ova. This view has not met with very much support, and in the very young marsupial their distribution and histological appearance are entirely against such an interprepation. They lie more deeply than the superficially situated primordial ova, and do not in the least resemble them. Schrön (42) derives them from the lutein elements of old corpora lutea, but it will be readily seen (Pl. 40, fig. 13) that there is a well-marked difference between the cells of an old corpus luteum and interstitial cells in marsupials. Rabl (36) sees in them modified elements of the theca interna and of atresic follicles, but among the marsupials the theca interna cells are not so well developed as in higher mammals, and do not resemble interstitial cells. Benthin (5) thinks they are derived from the conjunctive cells of the ovary, but only after these elements have been differentiated into atresic follicles (faux corps jaunes); this is also the view of Cohn (8) and Limon (21).

A number of recent observers—Sainmont (39), Regaud et Policard (38), Athias (3), etc.—maintain that the interstitial cells originate by the transformation of conjunctive cells. Popoff (34) admits this in the dog, but in the mole and weasel thinks they come from the theca interna cells after these have formed an atresic follicle. Another very prevalent opinion is that held by Kingsbury (18) and Van der

Stricht (49), who regard the interstitial tissue as derived in the first place from stroma cells, since it is to be found before the formation of follicles, and consider that it is later added to by cells derived from atresic follicles. Limon (loc. cit.) considers that when once these cells are formed they do not undergo any alteration. Regaud and Dubreuil (37) point out that this would lead to an enormous increase of the tissue, and conclude that it is necessary for the old cells to be removed and consequently recognise stages in their degeneration.

Turning now to the marsupials, we find that only one follicle appears to burst at a time in P. cinereus, but it is possible to find two or sometimes even three corpora lutea in the ovary at the same time, one being active and the others old. It seems legitimate to assume from this that they represent the corpora formed at the one or two preceding ovulations. The corpus of the immediately preceding ovulation is often more eosinophile than the active gland, but the still older one is less so. The older ones, too, are not so sharply marked off from the surrounding stroma by a connective tissue theca. They appear to be invaded by the stroma and cut up into pieces. This is marked in P. cinereus, and care is necessary to distinguish a gland in this state from a collection of groups of interstitial cells, but there is always a difference between them. A somewhat similar condition obtains in P. mitchelli, but the old corpora lutea do not become so cut up, and always appear to retain a connective tissue sheath and to undergo complete degeneration therein. In the remaining species where old corpora lutea were examined, among them T. vulpecula, it is almost certain that the old lutein cells degenerate in situ and that their remains are removed by phagocytosis, as described by Sandes in Dasyurus viverrinus (40). The evidence, then, is strongly against the assumption that lutein cells are transformed into interstitial cells.

In the one or two cases in P. cinereus, P. mitchelli, and T. vulpecula where atresic follicles are present the

cells are hypertrophied, and to a certain extent resemble interstitial cells, but I have no undoubted indication of any modification from one to the other, and the two can always be distinguished. As pointed out above, Limon states that the interstitial cells, after they are formed, persist and undergo no noticeable alteration, and this certainly seems to be true in the marsupials. Certain objections to the acceptance of this fairly widely advocated theory of the transformation of the cells of the atresic follicle are obvious. In the first place, it would be extremely difficult to bring forward conclusive evidence of such a change; the evidence so far adduced is far from convincing, and my own observations lead me to think that it does not occur in the Marsupialia. Secondly, if the interstitial tissue were added to indefinitely in this way, there would be an enormous difference between the ovary of the young and old mature female, which is not the case, and a striking increase in the size of the ovary with age. Regaud and Dubreuil (loc. cit.) see this and say that therefore there must be degeneration in the interstitial cells. Sainmont (39) actually goes so far as to distinguish degeneration stages. But surely if there is interstitial tissue to start with, and the atresic follicle is a product of the degeneration of the Graafian follicle, it is more natural to suppose that these degeneration stages are simply the end of the breakdown of the follicle, and that the true interstitial cells are untouched. In other words, a more straightforward explanation of these phenomena seems to be that during atresia the cells of the follicle pass through a stage in which they resemble interstitial cells.

Sections of the ovaries of two specimens of pouch young of T. vulpecula were examined, and it was found that even in the young still attached to the teats interstitial cells were present. The ova and follicles were in very early stages of development, in many cases the follicle was not formed around the ovum, and in no case was the theca folliculi formed. A number of groups of polygonal interstitial cells in a non-active condition were found situated in

the inner part of the ovary. These could not possibly be regarded as coming from an old corpus luteum or an atresic follicle, for neither structures could yet have been formed, nor were they derived from a theca interna, as this had not yet been laid down.

A similar observation has been made by v. d. Broek (**7a**) on a pouch-young of Sminthopsis crassicaudatus, 25 mm. in length, in which interstitial tissue was present, and he concludes: "So meine ich, dass die Entwicklung dieser Drüse, bei Beutlern ohne jeden Zusammenhang mit dem Alter der Tiere ist, auch nichts mit der Bildung von Corpora lutea und atretischen Follikeln zu thun hat"; a conclusion in entire agreement with my own, although v. d. Broek's paper was not known to me when I arrived at it. A number of authors, among others Van der Stricht (**49**), Allen (**2**), and Aimé (**1**), describe these cells in the ovaries of embryos of various species of mammals.

The interstitial tissue, then, is undoubtedly present at such an early stage that it must have originated in the stroma of the ovary independently of the theca or its derivatives, whether corpora lutea or atresic follicles, and is to be regarded as a tissue sui generis, perhaps derived from the stroma cells. In the Marsupialia I have not found anything to indicate that it is added to by cells from the theca interna, degenerating corpora lutea, or atresic follicles; indeed, such evidence as is forthcoming is against any of these interpretations. The tissue persists without degenerating and undergoes slight hypertrophy during the periods of ovarian activity.

Interstitial cells are always easily distinguishable from ordinary stroma cells (Pl. 40, fig. 13). They are polygonal instead of elongated and fibrous, and possess a typical vesicular nucleus with a nucleolus. The chromatin is more scattered than in the long nuclei of the cells of the stroma. When stained their nuclei are much lighter in colour and their cytoplasm takes eosin more readily. If in small numbers they appear, under a low magnification, as pale reddish

islands scattered in the general body of the stroma, and when present in large quantities they form the bulk of the substance of the ovary through which the stroma cells pass in interlacing strands.

This interstitial tissue takes part in the general ovarian activity during pregnancy, or, rather, subsequent to ovulation. The individual cells and their nuclei undergo hypertrophy, although they never become so large as the cells of the corpora lutea. This enlargement is very similar to that described by Lane-Claypon (19) in the rabbit, and the masses of active interstitial cells in the marsupial ovary are very similar in appearance to those in the rabbit. I have been unable to obtain the slightest evidence to show that in any marsupial certain of these cells situated near the periphery pass through a form of ovogenesis and become true ova, as is stated to occur in the rabbit (Lane-Claypon, loc. cit., p. 55). On the contrary, it is always perfectly easy to distinguish them from the oocytes at all stages, and careful searching has failed to reveal any cells that might be interpreted as intermediate stages.

When fully hypertrophied the interstitial masses present the appearance of typical epitheloid glandular tissue in a state of activity. Small chromagen granules are scattered through them, and their fatty or lipoid globules are dissolved out by the xylol so that they resemble lutein cells in appearance. They are plentifully supplied with blood-capillaries, which ramify through them in all directions. It seems beyond dispute that they are to be regarded as masses of secretory tissue. Their maximum enlargement is reached about the time that the corpus luteum becomes fully formed. Although they somewhat resemble the latter in transverse section, the difference between the two tissues becomes obvious when a strip of interstitial tissue comes to lie quite close to an active corpus luteum. In a similar manner the difference between interstitial cells and those of a degenerating corpus may also be seen at a glance (Pl. 40, fig. 13).

It has been noted in the rabbit that interstitial cells appear

to remain active for a longer time than those of the corpus luteum, and are in a fully active condition long after the birth of the young, when the corpus luteum has begun to decline. This statement appears to hold good also for those marsupials in which the tissue is present.

A very remarkable fact, that had not previously been noticed, came to light when making a list of the marsupial ovaries from the point of view of the quality and quantity of interstitial tissue they contained. It was found that they could be divided into two groups, according to whether this tissue was present or absent, as follows:

Interstitial tissue present.	Interstitial tissue absent.
1. Macropus ualabatus.	1. Perameles obesula.
2. Macropus ruficollis.	2. Perameles nasuta.
3. Macropus dorsalis.	3. Dasyurus maculatus.
4. Macropus thetidis.	4. Dasyurus viverrinus.
5. Macropus parma.	5. Didelphys aurita.
6. Petrogale penicillata.	6. Metachirus nudicaudatus.
7. Onychogale frenata (?).	
8. Trichosurus vulpecula.	
9. Phascolarctos cinereus.	
10. Phascolomys mitchelli.	

It will be seen at once that this grouping corresponds with the two main divisions of the Marsupialia, namely, the group Polyprotodontia and the group Diprotodontia. In the former the interstitial tissue is absent and in the latter it is present.

Four other authors have recently examined single ovaries from certain species of marsupials. Benthin (**5**) describes very briefly the ovaries of Pseudochirus (sp.?) and Phalanger orientalis. The latter ovary is admittedly badly preserved, and in neither case, apparently, was attention paid to the interstitial tissue, as the investigator was concerned almost entirely with follicular atresia.

v. d. Broek (**7a**), in a work on the development of the urogenital system of marsupials, states that he has found an "interstitial gland" in the ovaries of adult females of

Petrogale penicillata, which agrees with my own results, in a young female of Halmaturus (Macropus) Derbianus, and in a pouch-young of Sminthopsis crassicaudatus, 25 mm. long. All three species belong to the group Diprotodontia.

Schaeffer (41) investigated in particular the interstitial "gland" in a large series of fifty mammals, including the marsupial Macropus melanops (sic! M. giganteus var. melanops) and Petrogale penicillata. Of the last it is stated that it possesses "eine typische interstitielle Eierstocksdrüse," a finding quite in agreement with my observations. In regard to M. melanops it is stated that "Eine glande interstitielle ist demnach nicht vorhanden." This positive assertion appears to be open to grave doubt, for in all the other Macropodidæ examined, not only is interstitial tissue present, but a large quantity of it is to be found. Moreover, the author himself describes in this very ovary a tissue of which he writes: "Er besteht aus kleineren zellgruppen, die durch zahlreiche Kapillaren von einander getrennt werden. Die Zellen sind polygonal, messen 10 μ in Durchmesser, die Kerne sind 8 μ gross mit einem Kernkörperchen." This, however, is a sufficiently accurate description of the interstitial tissue in a non-active condition and, at any rate, does not apply to the ordinary stroma cells. The author admits that it is "drusenähnlich," but states that it cannot be considered as interstitial because it occurs near the hilus and is marked off from the tissue containing the egg-cells. This certainly does not appear to be a sufficient reason for not regarding it as interstitial tissue, which may occur in any part of the ovary, and I am of the opinion that it is better to regard it as interstitial tissue until it can be shown to be something different, and that apparently unique in the marsupial ovary. The ovary used was preserved in 5 per cent. formol, a fluid that gives very bad fixation of ovarian tissue.

Fraenkel (10) examined a series of forty-five mammals, six of which were marsupials. Three of these, i. e. Halmaturus (Macropus) thetidis, Petrogale penicillata, and

Onychogale frenata, I have also examined, and our results are in agreement: interstitial tissue is present in the ovary. Another species, Phascolomys latifrons, is closely allied to P. mitchelli, and in this too he reports the presence of interstitial tissue. In the remaining two species, namely, Halmaturus (Macropus) giganteus and Macropus sp., he states that such tissue is absent, but in both these cases his evidence is quite unsatisfactory. Of one, H. giganteus, he does not say how the material was preserved, and admits with regard to it, "Es muss also durch pathologische Veränderung oder Conservirungsfehler das Organ geschädigt worden sein," yet still uses it for histological purposes. Moreover, as we have seen above, Schaeffer describes in the ovary of the same species cells that are almost undoubtedly interstitial cells. The other, Macropus sp., was preserved in 3 per cent. formol, a bad fluid for histological work, and the sections employed were 40 μ thick!

It is to be regretted that in investigations of this kind better fixatives were not employed throughout, and this applies particularly to Schaeffer's work, in which formol was generally used. Also it is to be noted that no account was taken of the condition of the animal in regard to pregnancy or œstrus, and these are important points, as the tissue undergoes well-marked hypertrophy during these periods of functional activity. The failure to take into account the state of activity of the reproductive system, and to use suitable fixing fluids, detracts greatly from the value of both papers; and, indeed, in order to be certain of the absence of this tissue it is necessary to examine serial sections of one or preferably a series of pregnant animals.

With the possible exception of Macropus sp. ?, which cannot be admitted until it has been more satisfactorily established, we may say of the marsupials up to the present examined that the Diprotodontia possess and the Polyprotodontia lack interstitial cells.[1]

[1] It is interesting in this connection to call attention to the observations of Symmington, confirmed more recently by Fraser and Hill (13),

The amount of tissue present in the ovary is not the same in all species of Diprotodontia. It is least marked in Phascolomys mitchelli, and, indeed, in a previous investigation (31) it was stated: "Das interstitielle Gewebe des Ovariums scheint bei P. wombat (i.e. mitchelli) ganz zu fehlen." Re-examination of the sections, however, shows that the tissue is present, but not in nearly such large masses as in the other species. Small clumps of interstitial cells are to be found here and there in the stroma. When the sections were examined previously the only ovaries available for comparison were those of M. ruficollis and P. penicillata, and these happen to be the two species in which the tissue is most abundantly present. It forms half or more of the entire ovary, and is easily recognisable in sections with the naked eye; in comparison with this the small amount in P. mitchelli was overlooked. With the exception of this last species all the Diprotodontia examined have a good deal of interstitial tissue which appears to reach its maximum in the Macropodidæ and culminates in P. penicillata, where it forms at least half the total bulk of the ovary, or if the corpus luteum be excluded, by far the greater part of the remaining ovarian tissue.

No trace of similar interstitial cells was found in the Polyprotodontia even in pregnant animals. The stroma here is composed almost entirely of ordinary elongated spindle-shaped stroma cells with their long- drawn-out nuclei. In it may be found a few very small groups of cells that are not so elongated as the remainder, but they are not in the least like the interstitial cells of the Diprotodontia. They are not so large, not polygonal, have not spherical nuclei, do not react

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for, although they have no bearing on the main points of this paper, they instance an analogous difference in the distribution of glandular tissue in the two sub-groups of the Marsupialia. These authors have found that Diprotodonts possess a very large and highly developed cervical thymus which is entirely absent in Polyprotodonts. At present it does not seem possible to advance any satisfactory theory to explain such differences in the occurrence of the various glandular tissues.

in the same way to stains, and, most important of all, they do not undergo hypertrophy during pregnancy.

It is not within the scope of this paper to deal at any length with the physiological import of the interstitial tissue, since no physiological observations have been made on the animals examined, and physiological deductions from purely histological data are often of little value. [A fairly full review of the physiological aspect of this subject is given by Schaeffer (**41**)]. Conversely, however, histological and anatomical observations must not be disregarded when the functions of a structure are being considered.

The marked influence of the ovary upon the general metabolism of the body of the female has long been recognised, and of late years evidence has been accumulated to show that this influence is not of a nervous character but of the nature of an internal secretion or hormone. Part of this influence has been held to be due to the corpus luteum (**30**), and recently a certain amount of it has been ascribed to the interstitial cells. Marshall (**26**) has come to the conclusion that the maturation of the ovum and the phenomenon of pro-œstrum and œstrus are the result of a factor whose origin is to be sought in the interstitial cells. A few lines previously he mentions that "epitheloid interstitial cells" are numerous in the stroma, so that there can be no doubt he is referring to ordinary interstitial cells as dealt with above. It is obvious that this explanation is insufficient, or, at any rate, not of general application, for we cannot look to these cells for the stimulus in the animals where such cells do not exist, as, for example, in the Polyprotodontia.

Bouin and Ancel (**7**), partly from their own observations and partly from those of previous writers, suggest that mammals may be divided into two classes. Those which possess two kinds of corpus luteum, one periodic (i.e. C. L. périodique), and the other that of pregnancy (i.e. C.L. gestatif). The other group includes those animals which only ovulate after copulation, and consequently have only the corpus luteum of gestation. They state further that only the

second group possess an interstitial "gland," and deduce from this that the gland replaces functionally the periodic corpus luteum.

If this be so, why is it that the interstitial tissue appears in its most active condition when corpora lutea are present in the ovary? Further, it has been pointed out by Frank (12) that guinea-pigs, white rats and cats ovulate spontaneously and yet possess an interstitial "gland." In examples of M. ruficollis and P. mitchelli, probably non-pregnant, corpora lutea were well developed. These may constitute further examples of animals ovulating spontaneously and yet possessing interstitial tissue. Jardry (17) maintains that the secretion of this "gland" has a general trophic influence. He says: "Elle règle, accelère la nutrition intime des tissues, en agissant avec prédilection sur le système génital d'une part, et d'autre part sur l'assimilation des albuminoides et des phosphates au sein des tissues."

The general criticism made by Fraenkel (10) and later by Kingsbury (18) on all such théories as those mentioned above, seems to apply with even greater force when we consider the marsupials. This latter author says (p. 79) that we should expect "a gland existing for the specific purpose of forming substances of very distinct value to the organism as a whole would be constant in its presence and development." He points out that there is great variability in these matters, and Schaeffer (41) and Fraenkel (10) have further emphasised this variability by an examination of the ovaries of large series of different species of mammals. It has been pointed out above that within the limits of the group Diprotodontia there is marked variation in the number of interstitial cells present, it may be enormous, or, on the other hand, only a comparatively few small groups of such cells are to be found. In the Polyprotodontia, as we have seen, such cells are absent altogether.

At present there appears to be no satisfactory function assigned to these interstitial cells, and any theory put forward, in order to be completely satisfactory, must be able

to show why it is necessary for certain animals to possess the cells and yet not necessary in other animals that do not appear to have any compensating structure.

In spite of the fact that these cellular masses present such a typically glandular appearance, I have endeavoured throughout to avoid the use of the term gland because its use hardly seems justifiable. Used in a morphological sense, the word gland implies a certain definiteness and constancy, and these are not exhibited by the interstitial tissue. Physiologically, also, the word gland is currently used to denote a structure secreting a substance that plays some assignable rôle in the vital activity of the organism. The interstitial cells probably produce a secretion of a fatty or lipoid nature, but whether this has any influence on the whole or any part of the female has yet to be shown. The term interstitial cells or tissue does not imply so much as gland, and is preferable in the present state of our knowledge.

Summary.

The Corpus Luteum.

(a) Follicular Wall.—The membrana granulosa in the three species, P. cinereus, T. vulpecula, and D. aurita, is composed of typical polygonal cells arranged three or four cells deep around the ripe follicle. The theca folliculi also calls for no special comment in any case. It is composed of internal and external layers, does not contain any included interstitial cells, and its cells are always readily distinguishable from membrana granulosa cells.

(b) The Formation of the Corpus Luteum.—The corpus luteum in P. cinereus is formed by the irruption of both layers of the theca folliculi, which burst through the membrana granulosa and form a lining on its inner side. This method of formation is similar to that in P. obesula, P. nasuta, and M. ruficollis. The ripe follicle in T. vulpecula collapses when the ovum is extruded, and the

central cavity is at once obliterated. The theca folliculi is drawn in with the membrana granulosa, which it penetrates, and the connective tissue becomes irregularly distributed through the body. It is unlike the process in any other marsupial so far examined, but to a certain extent resembles that in the mouse.

In D. aurita the thecal irruptions do not at once go through the membrana granulosa, but push it before them until the central cavity is practically filled in, and then they break through and form the central plug of connective tissue. In one example, a very early stage, mitoses were found in the cells of the membrana granulosa, as was also the case in P. obesula and P. nasuta.

(c) The fully formed Corpus Luteum.—The corpus luteum in P. cinereus remains hollow even when fully grown, and the central cavity does not get filled in until some time after the birth of the young, apparently not until the gland has started to decline. This condition is apparently unique.

In T. vulpecula the corpus luteum is fairly typical when full grown, save that its connective tissue is much more irregularly arranged than in other marsupials.

The condition of the corpus in D. aurita is very similar to that in D. viverrinus.

In no case is the membrana granulosa shed, nor does the theca interna contribute to the lutein cells of the corpus luteum.

The Interstitial Tissue.

There is present in the ovary of certain species of marsupials a tissue which corresponds histologically to the interstitial tissue in the ovary of the higher mammals. The cells are always distinguishable from ordinary stroma cells, cells of the theca interna, old lutein cells, or the cells of an atresic follicle, and there is no evidence that any of the last three are at any time transformed into interstitial cells.

Such cells are present in the pouch young of T. vul-

pecula before they could have been derived from any of the sources suggested above.

Interstitial tissue is to be regarded as a tissue sui generis, although it is possible that it may originate from modified stroma cells at a very early stage.

The tissue is irregularly distributed in the various species of marsupials, and it is worthy of note that it is present in all the Diprotodontia and absent in the Polyprotodontia so far examined. It may be present only as a few small groups of cells or in such quantity as to form by far the largest part of the bulk of the ovary, excluding corpora lutea, as, for example, in P. penicillata.

The tissue has a typical glandular appearance, but no satisfactory account of its function has yet been put forward, and in view of this and its irregularity it is preferable not to call it a gland, but retain the term interstitial tissue or cells.

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EXPLANATION OF PLATE 40,

Illustrating Mr. Chas. H. O'Donoghue's paper, "On the Corpora lutea and Interstitial Tissue of the Ovary in the Marsupialia."

Fig. 1.—Photomicrograph (× circa 6). Transverse section of the ovary of Phascolarctos cinereus (No. 2), showing an early stage in the formation of the corpus luteum. Atresic follicles are present and the islets of interstitial tissue are clearly visible in the ovarian stroma.

Fig. 2.—Photomicrograph (× circa 6). Transverse section of the corpus luteum of P. cinereus (No. 5). The large size is clearly shown, and the characteristic hollow form is retained although the embryos are well developed and 9 mm. in length.

Fig. 3.—Photomicrograph (× circa 6). Transverse section of the corpus luteum of P. cinereus (No. 9). The embryos are in a late uterine stage, shortly before birth, but the corpus is still hollow and about at its maximum size.

Fig. 4.—Photomicrograph (× circa 12). Transverse section of the corpus luteum of Trichosurus vulpecula (No. 2). An early stage in the formation, showing the way in which the follicle collapses completely and even becomes partly inverted and draws the theca in with it.

Fig. 5.—Photomicrograph (× circa 12). Transverse section of the corpus luteum of T. vulpecula (No. 5). A slightly later stage than fig. 4. The very irregular distribution of the connective and luteal tissue is clearly shown.

Fig. 6.—Photomicrograph (× circa 12). Transverse section of the corpus luteum of T. vulpecula (No. 6). The fully formed corpus is irregular and protruding, and three fairly ripe follicles are shown.

Fig. 7.—Photomicrograph (× circa 12). Transverse section of the corpus luteum of T. vulpecula (No. 12). The maximum size of the corpus accompanying late uterine embryos. The irregular form is shown, and also the manner in which the body projects from the ovary.

Fig. 8.—Photomicrograph (× circa 8). Transverse section of the entire ovary of Didelphys aurita (No. 6). Parts of twelve fully formed, but not fully grown, corpora lutea are present in the section. Comparison with fig. 1 brings out the difference between the ovary of this species and of P. cinereus. Interstitial tissue is absent.

Fig. 9.—Photomicrograph (× circa 40). Transverse section of an early stage in the formation of the corpus luteum in D. aurita (No. 2). The connective tissue ingrowths have pushed the membrana granulosa cells inwards, but not yet broken through it.

Fig. 10.—Photomicrograph (× circa 40). Transverse section of a later stage in the formation of the corpus luteum in D. aurita (No. 4). The whole structure has enlarged and the connective tissue ingrowths have broken through into the central cavity.

Fig. 11.—Photomicrograph (× circa 40). Transverse section of a still later stage in the formation of the corpus luteum in D. aurita (No. 3). The corpus is almost fully formed and the central cavity nearly obliterated.

Fig. 12.—Photomicrograph (× circa 40). Transverse section of fully formed and grown corpus luteum in D. aurita (No. 5). The whole structure is very similar to a corpus luteum in Dasyurus viverrinus.

Fig. 13.—Photomicrograph (× circa 40). Transverse section of a portion of the ovary of Macropus dorsalis. This shows at the same time, A, part of an active corpus luteum, C, part of an old corpus luteum, and B, active interstitial tissue in the ovarian stroma between them. The differences between the three kinds of tissue are obvious.